Litton BIONETICS

Mutagenicity Evaluation of Compound FDA 75-90 (Potassium Bicarbonate)
Final Report
7/77

MUTAGENICITY EVALUATION
OF
FDA 75-90
POTASSIUM BICARBONATE

FINAL REPORT

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FINAL REPORT

SUBMITTED TO

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LBI PROJECT NO. 2672

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Litton BIONETICS

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EVALUATION SUMMARY

The test compound, FDA 75-90, Potassium Bicarbonate, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: July, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound: FDA 75-90, Potassium Bicarbonate

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: December 29, 1976

2. Description: White crystals

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535, TA-1537, TA-1538, TA-98, TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 75-90, Potassium Bicarbonate

2. Test solvent: * Saline

3. Solubility of the test compound under treatment conditions: Soluble

4. Additional comments: White crystals

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: February 21, 1977

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	0.0395	0.825
1/2 50% Survival	0.0790	1.650
50% Survival	0.1580	3.300

*The concentration of solvent was equal to the highest volume of test material added.

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000298146

B. TEST DATE: MARCH 2, 1977

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE									
				TA-1535		TA-1537		TA-1538		TA-98		TA-100	
				1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION													
SOLVENT CONTROL*		---	---	30	34	19	14	21	16	23	14	191	142
POSITIVE CONTROL**		---	---	>1000	304	>1000	>1000	472	>1000	>1000	>1000	498	461
TEST	0.15800 %	---	---	33	34	16	15	18	18	29	16	280	244
	0.07900 %	---	---	46	38	18	11	11	15	26	15	270	250
	0.03950 %	---	---	43	53	12	10	15	12	14	28	175	278
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	29	33	27	26	20	28	27	56	289	258
		RAT	LIVER	37	36	34	23	14	23	41	34	272	264
		MONKEY	LIVER	35	28	28	34	15	11	28	21	361	414
POSITIVE CONTROL***		MOUSE	LIVER	117	536	180	154	409	487	347	381	>1000	>1000
		RAT	LIVER	99	220	212	185	450	449	485	356	867	>1000
		MONKEY	LIVER	669	626	182	189	434	410	421	288	>1000	>1000
TEST	0.15800 %	MOUSE	LIVER	35	29	25	34	25	22	38	68	240	272
	0.07900 %	MOUSE	LIVER	18	23	31	22	19	18	27	49	289	278
	0.03950 %	MOUSE	LIVER	18	25	31	29	10	17	42	38	266	279
	0.15800 %	RAT	LIVER	24	14	19	21	17	31	35	45	206	235
	0.07900 %	RAT	LIVER	19	12	10	17	31	16	32	35	278	292
	0.03950 %	RAT	LIVER	11	17	15	10	11	18	38	32	297	299
	0.15800 %	MONKEY	LIVER	33	28	24	37	25	24	43	34	294	291
	0.07900 %	MONKEY	LIVER	22	35	15	17	16	30	43	33	232	285
	0.03950 %	MONKEY	LIVER	24	36	34	22	24	15	31	26	251	245

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
TA-1537 QM 20 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

- INDICATES NO DATA WAS TAKEN.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/14/77

NONACTIVATION COMPOUND 000298146

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		338.20	12.73	14.11	11.85	9.80	16.81	13.67	CONTROLS
NAP		580.47	142.32	99.71	120.00	195.21	216.15	189.62	
NA1		251.39	21.21	8.13	2.72	11.77	15.55	8.58	TEST DATA
NA2		242.34	11.99	10.33	2.89	7.94	8.68	5.70	
NA3		177.63	11.38	12.06	4.47	7.94	11.54	7.15	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/14/77

SPECIES ICRFLO/MOUSE COMPOUND 000298146

TEST	ORG	TA100 HIS EX-R	TA1535 HIS EX-R	TA1537 HIS EX-R	TA1538 HIS EX-R	TA98 HIS EX-R	00000D4 ADE EX-5	00000D4 TRY EX-5	
ACT	A+C	78.27	6.72	9.23	10.74	9.30	5.09	8.16	NEGATIVE CONTROLS
ACT	A-C	68.18	10.10	6.99	7.24	8.35	8.21	4.36	
ACT	ALI	92.47	12.40	10.09	10.51	10.02	15.23	6.53	
ACT	ALH	129.25	11.26	10.80	11.33	11.51	7.66	5.92	
ACT	PLI	141.76	209.75	69.01	668.98	126.93	203.20	76.46	POSITIVE CONTROLS
ACT	PLH	114.03	13.36	18.48	18.52	42.01	52.64	8.31	
ACT	LI1	47.46	7.58	15.71	8.45	19.10	16.47	3.90	TEST COMPOUND
ACT	LI2	63.92	9.29	18.41	9.82	19.08	9.34	3.89	
ACT	LI3	28.28	7.55	19.42	11.55	21.25	6.59	4.15	
ACT	LU1	26.97	7.86	21.49	7.91	14.97	23.07	4.29	
ACT	LU2	47.67	11.53	19.88	6.74	16.16	11.67	4.81	
ACT	LU3	80.81	13.05	19.37	9.89	19.58	8.00	3.71	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/14/77

SPECIES SPRDAW/RAT COMPOUND 000298146

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	76.96	6.17	8.43	4.62	14.02	18.42	4.59	NEGATIVE CONTROLS
ACT	A-C	64.02	3.41	6.23	6.66	14.18	11.04	2.59	
ACT	ALI	76.31	6.98	8.14	11.94	11.29	19.58	5.60	
ACT	ALU	92.59	5.85	10.86	11.70	12.36	21.12	5.71	
ACT	PLI	225.23	329.92	91.38	122.04	135.13	63.49	68.82	POSITIVE CONTROLS
ACT	PLU	96.88	5.65	6.70	180.65	179.14	15.03	5.01	
ACT	LI1	63.36	5.43	13.17	8.45	20.70	6.59	3.54	TEST COMPOUND
ACT	LI2	42.99	4.25	12.09	9.49	16.45	12.54	3.54	
ACT	LI3	75.42	4.55	10.46	5.88	21.66	3.86	1.82	
ACT	LU1	52.44	4.44	25.51	4.13	22.41	10.17	5.09	
ACT	LU2	70.87	4.82	23.92	11.48	34.78	14.46	4.78	
ACT	LU3	70.95	6.30	20.92	8.21	22.54	14.39	7.91	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/14/77

SPECIES RHESUS/MONKEY COMPOUND 000298146

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	66.44	9.89	7.04	18.56	4.03	14.83	4.84	NEGATIVE CONTROLS
ACT	A-C	52.30	7.21	4.84	15.04	2.11	10.34	2.48	
ACT	ALI	34.39	8.99	8.47	20.04	6.60	15.43	12.92	
ACT	ALU	35.53	5.99	7.10	18.40	3.80	18.41	8.20	
ACT	PLI	81.25	149.45	26.81	201.96	270.39	36.48	56.77	POSITIVE CONTROLS
ACT	PLU	34.77	10.56	30.49	17.07	5.15	16.21	5.60	
ACT	LI1	46.04	6.87	6.30	12.87	6.35	13.30	7.50	TEST COMPOUND
ACT	LI2	35.73	7.77	5.65	8.04	5.71	11.03	5.45	
ACT	LI3	31.00	6.85	3.33	9.18	5.98	12.82	7.68	
ACT	LU1	36.78	6.03	5.22	6.23	3.83	15.15	11.93	
ACT	LU2	45.87	6.33	7.95	15.07	5.06	12.90	5.88	
ACT	LU3	35.21	10.30	6.73	11.68	3.12	6.20	6.29	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU or A+T = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (Macaca mulatta)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 75-90, Potassium Bicarbonate, was evaluated for genetic activity in a series of *in vitro* microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate tests

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, FDA 75-90, Potassium Bicarbonate, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

David J. Brusick 7/29/77

David J. Brusick, Ph.D. Date
Director
Department of Molecular Toxicology

Reviewed by:

Robert J. Weir 7/29/77

Robert J. Weir, Ph.D. Date
Vice President

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagnes to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the *his*$^-$ cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

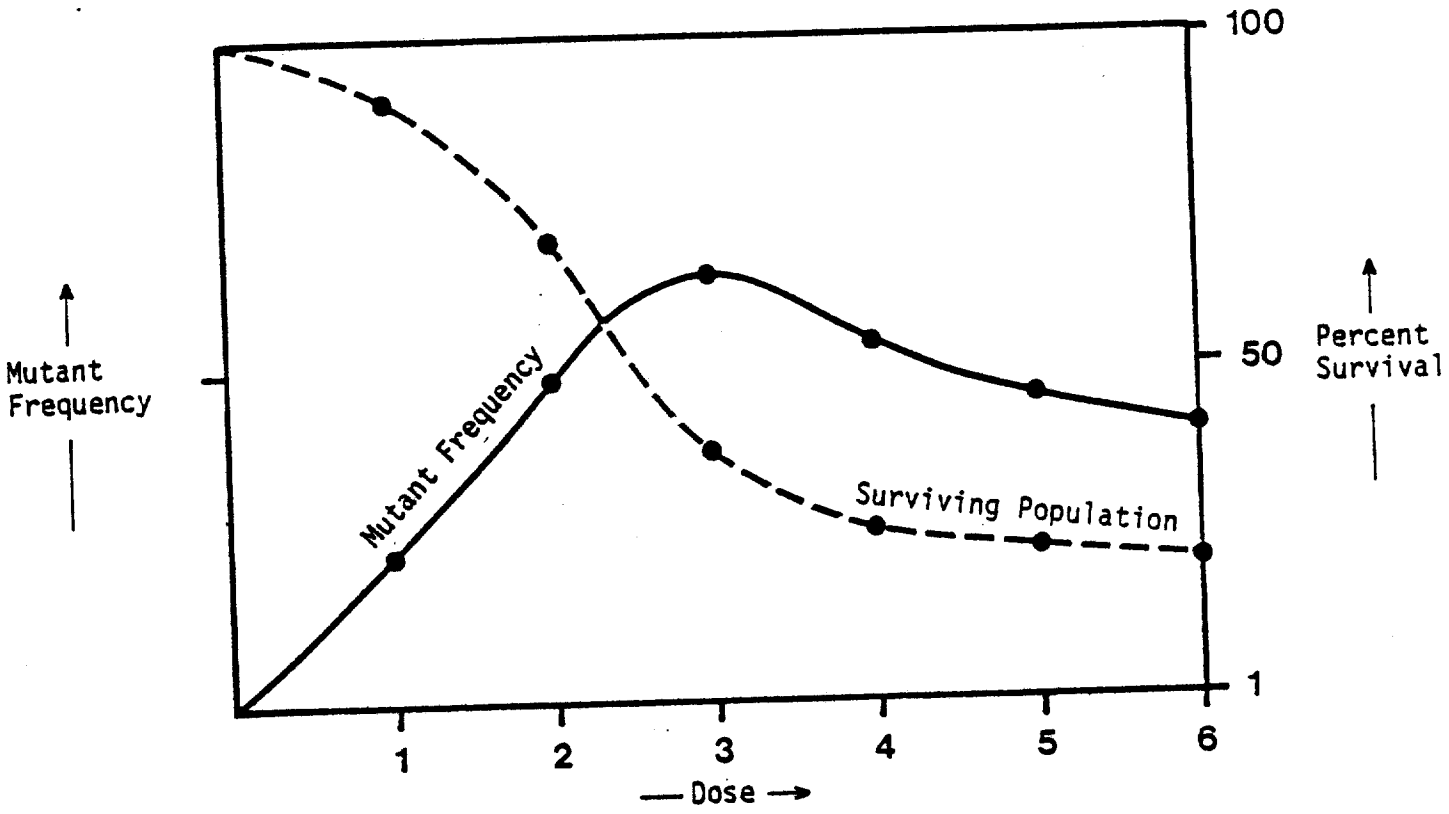
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT

(1) Dose levels 1,2 & 3 were used

(2) Dose levels 2, 3 & 4 were used

(3) Dose levels 3, 4 & 5 were used

OBSERVED DOSE RESPONSE

A typical positive dose response set of data would be obtained.

The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.

Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672 DATE - 07/14/77
EXPERIMENT 706706 DETECTOR TA100 SPECIES /

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0445	1505	338.20	0
	NAP		EMS 0.066%	0773	4487	580.47	0
00029R146	NA1		0158-3 PCT.	0790	1986	251.39	0
00029R146	NA2		0079-3 PCT.	0718	1740	242.34	0
00029R146	NA3		0395-4 PCT.	1073	1906	177.63	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706705 DETECTOR TA1535 SPECIES / DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0385	0049	12.73	0
	NAP		EMS 0.2%	0586	0834	142.32	0
00029A146	NA1		0158-3 PCT.	0264	0056	21.21	0
00029A146	NA2		0079-3 PCT.	0367	0044	11.99	0
00029A146	NA3		0395-4 PCT.	0492	0056	11.38	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672 DATE - 07/14/77
EXPERIMENT 706601 DETECTOR TA1537 SPECIES /

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	1247	0176	14.11	0
	NAP		QM 13 UG/ML	0341	0340	99.71	0
000298146	NA1		0158-3 PCT.	1795	0146	8.13	0
000298146	NA2		0079-3 PCT.	1297	0134	10.33	0
000298146	NA3		0395-4 PCT.	1327	0160	12.06	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672 DATE - 07/14/77
EXPERIMENT 709602 DETECTOR TA1538 SPECIES /

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0405	0048	11.85	0
	NAP		NF 667 UG/ML	0405	0486	120.00	0
000298146	NA1		0158-3 PCT.	0478	0013	2.72	0
000298146	NA2		0079-3 PCT.	0485	0014	2.89	0
000298146	NA3		0395-4 PCT.	0492	0022	4.47	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672 DATE - 07/14/77
EXPERIMENT 706707 DETECTOR TA98 SPECIES /

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0306	0030	9.80	0
	NAP		NF 667 UG/ML	0876	1710	195.21	0
000298146	NA1		0158-3 PCT.	0603	0071	11.77	0
000298146	NA2		0079-3 PCT.	0856	0068	7.94	0
000298146	NA3		0395-4 PCT.	0705	0056	7.94	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672 DATE - 07/14/77
EXPERIMENT 711805 DETECTOR 0000D4 SPECIES /

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	1178	0198	0161	16.81	13.67	0
	NAP		EMS 1.0 %	0260	0562	0493	216.15	189.62	0
000298146	NA1		0033-1 PCT.	0688	0107	0059	15.55	8.58	0
000298146	NA2		0165-2 PCT.	1106	0096	0063	8.68	5.70	0
000298146	NA3		0825-3 PCT.	0979	0113	0070	11.54	7.15	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672 DATE - 07/14/77
EXPERIMENT 708802 DETECTOR TA100 SPECIES ICRFLO/MOUSE

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0833	0652	78.27	0
	A-C		SOLVENT	0682	0465	68.18	0
	ALI		TISSUE	0810	0749	92.47	0
	ALU		TISSUE	0530	0685	129.25	0
	ACP	LI	DMN 90 UM/ML	0546	0774	141.76	0
	ACP	LU	DMN 90 UM/ML	0556	0634	114.03	0
000298146	ACT	LI1	0158-3 PCT.	1336	0634	47.46	0
000298146	ACT	LI2	0079-3 PCT.	1031	0659	63.92	0
000298146	ACT	LI3	0395-4 PCT.	1648	0466	28.28	0
000298146	ACT	LU1	0158-3 PCT.	1817	0490	26.97	0
000298146	ACT	LU2	0079-3 PCT.	1181	0563	47.67	0
000298146	ACT	LU3	0395-4 PCT.	0787	0636	80.81	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706802 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0625	0042	6.72	0
	A-C		SOLVENT	0515	0052	10.10	0
	ALI		TISSUE	0492	0061	12.40	0
	ALU		TISSUE	0506	0057	11.26	0
	ACP	LI	DMN 90 UM/ML	0318	0667	209.75	0
	ACP	LU	DMN 90 UM/ML	0217	0029	13.36	0
00029A146	ACT	LI1	0158-3 PCT.	0686	0052	7.58	0
00029A146	ACT	LI2	0079-3 PCT.	0581	0054	9.29	0
00029A146	ACT	LI3	0395-4 PCT.	0768	0058	7.55	0
00029A146	ACT	LU1	0158-3 PCT.	0471	0037	7.86	0
00029A146	ACT	LU2	0079-3 PCT.	0529	0061	11.53	0
00029A146	ACT	LU3	0395-4 PCT.	0475	0062	13.05	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 705901 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1224	0113	9.23	0
	A-C		SOLVENT	1130	0079	6.99	0
	ALI		TISSUE	0872	0088	10.09	0
	ALU		TISSUE	0722	0078	10.80	0
	ACP	LI	AMQ 333 UG/ML	1097	0757	69.01	0
	ACP	LU	AMQ 333 UG/ML	0801	0148	18.48	0
000298146	ACT	LI1	0158-3 PCT.	1203	0189	15.71	0
000298146	ACT	LI2	0079-3 PCT.	1146	0211	18.41	0
000298146	ACT	LI3	0395-4 PCT.	1030	0200	19.42	0
000298146	ACT	LU1	0158-3 PCT.	1075	0231	21.49	0
000298146	ACT	LU2	0079-3 PCT.	0976	0194	19.88	0
000298146	ACT	LU3	0395-4 PCT.	0862	0167	19.37	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708803 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0633	0068	10.74	0
	A-C		SOLVENT	0898	0065	7.24	0
	ALI		TISSUE	0590	0062	10.51	2
	ALU		TISSUE	0547	0062	11.33	2
	ACP	LI	ANTH 67 UG/ML	0332	2221	668.98	0
	ACP	LU	ANTH 67 UG/ML	0583	0108	18.52	2
000298146	ACT	LI1	0158-3 PCT.	0817	0069	8.45	2
000298146	ACT	LI2	0079-3 PCT.	1049	0103	9.82	2
000298146	ACT	LI3	0395-4 PCT.	0840	0097	11.55	2
000298146	ACT	LU1	0158-3 PCT.	0670	0053	7.91	2
000298146	ACT	LU2	0079-3 PCT.	0757	0051	6.74	2
000298146	ACT	LU3	0395-4 PCT.	0657	0065	9.89	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706901 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1322	0123	9.30	0
	A-C		SOLVENT	1306	0109	8.35	0
	ALI		TISSUE	1298	0130	10.02	2
	ALU		TISSUE	1086	0125	11.51	0
	ACP	LI	ANTH 67 UG/ML	1051	1334	126.93	0
	ACP	LU	ANTH 67 UG/ML	0845	0355	42.01	0
00029A146	ACT	LI1	0158-3 PCT.	0932	0178	19.10	0
00029A146	ACT	LI2	0079-3 PCT.	0896	0171	19.08	0
00029A146	ACT	LI3	0395-4 PCT.	0753	0160	21.25	0
00029A146	ACT	LU1	0158-3 PCT.	1102	0165	14.97	0
00029A146	ACT	LU2	0079-3 PCT.	1194	0193	16.16	0
00029A146	ACT	LU3	0395-4 PCT.	0899	0176	19.58	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710903 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1789	0091	0146	5.09	8.16	0
	A-C		SOLVENT	1193	0098	0052	8.21	4.36	0
	ALI		TISSUE	1333	0203	0087	15.23	6.53	0
	ALU		TISSUE	1605	0123	0095	7.66	5.92	0
	ACP	LI	DMN 90 UM/ML	1627	3306	1244	203.20	76.46	0
	ACP	LU	DMN 90 UM/ML	1721	0906	0143	52.64	8.31	0
000298146	ACT	LI1	0033-1 PCT.	2076	0342	0081	16.47	3.90	0
000298146	ACT	LI2	0165-2 PCT.	1338	0125	0052	9.34	3.89	0
000298146	ACT	LI3	0825-3 PCT.	1351	0089	0056	6.59	4.15	0
000298146	ACT	LU1	0033-1 PCT.	1283	0296	0055	23.07	4.29	0
000298146	ACT	LU2	0165-2 PCT.	1371	0160	0066	11.67	4.81	0
000298146	ACT	LU3	0825-3 PCT.	1212	0097	0045	8.00	3.71	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708904 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0638	0491	76.96	0
	A-C		SOLVENT	0681	0436	64.02	0
	ALI		TISSUE	0688	0525	76.31	0
	ALU		TISSUE	0459	0425	92.59	0
	ACP	LI	DMN 90 UM/ML	0218	0491	225.23	0
	ACP	LU	DMN 90 UM/ML	0609	0590	96.88	0
000298146	ACT	LI1	0158-3 PCT.	0999	0633	63.36	0
000298146	ACT	LI2	0079-3 PCT.	1205	0518	42.99	0
000298146	ACT	LI3	0395-4 PCT.	0769	0580	75.42	0
000298146	ACT	LU1	0158-3 PCT.	1188	0623	52.44	0
000298146	ACT	LU2	0079-3 PCT.	0944	0669	70.87	0
000298146	ACT	LU3	0395-4 PCT.	1098	0779	70.95	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708801 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0664	0041	6.17	0
	A-C		SOLVENT	0822	0028	3.41	0
	ALI		TISSUE	0802	0056	6.98	0
	ALU		TISSUE	0598	0035	5.85	0
	ACP	LI	DMN 90 UM/ML	0264	0871	329.92	0
	ACP	LU	DMN 90 UM/ML	0797	0045	5.65	0
000298146	ACT	LI1	0158-3 PCT.	0405	0022	5.43	0
000298146	ACT	LI2	0079-3 PCT.	0565	0024	4.25	0
000298146	ACT	LI3	0395-4 PCT.	0638	0029	4.55	0
000298146	ACT	LU1	0158-3 PCT.	0360	0016	4.44	0
000298146	ACT	LU2	0079-3 PCT.	0456	0022	4.82	0
000298146	ACT	LU3	0395-4 PCT.	0524	0033	6.30	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672 DATE - 07/14/77
EXPERIMENT 709601 DETECTOR TA1537 SPECIES SPRDAW/RAT

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1341	0113	8.43	0
	A-C		SOLVENT	1284	0080	6.23	0
	ALI		TISSUE	0799	0065	8.14	0
	ALU		TISSUE	0746	0081	10.86	0
	ACP	LI	AMQ 333 UG/ML	0812	0742	91.38	0
	ACP	LU	AMQ 333 UG/ML	2104	0141	6.70	0
000298146	ACT	LI1	0158-3 PCT.	0562	0074	13.17	2
000298146	ACT	LI2	0079-3 PCT.	0769	0093	12.09	0
000298146	ACT	LI3	0395-4 PCT.	0736	0077	10.46	0
000298146	ACT	LU1	0158-3 PCT.	0392	0100	25.51	0
000298146	ACT	LU2	0079-3 PCT.	0439	0105	23.92	0
000298146	ACT	LU3	0395-4 PCT.	0478	0100	20.92	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 712512 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 07/14/77

COMPOUND	TEST	OPG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1429	0066	4.62	0
	A-C		SOLVENT	1217	0081	6.66	0
	ALI		TISSUE	0896	0107	11.94	0
	ALU		TISSUE	0684	0080	11.70	0
	ACP	LI	ANTH 67 UG/ML	1366	1667	122.04	0
	ACP	LU	ANTH 67 UG/ML	0713	1288	180.65	0
000298146	ACT	LI1	0158-3 PCT.	1372	0116	8.45	0
000298146	ACT	LI2	0079-3 PCT.	1033	0098	9.49	0
000298146	ACT	LI3	0395-4 PCT.	1411	0083	5.88	0
000298146	ACT	LU1	0158-3 PCT.	1961	0081	4.13	0
000298146	ACT	LU2	0079-3 PCT.	0671	0077	11.48	0
000298146	ACT	LU3	0395-4 PCT.	0913	0075	8.21	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708901 DETECTOR TA98 SPECIES SPRDAW/RAT DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0906	0127	14.02	0
	A-C		SOLVENT	0931	0132	14.18	0
	ALI		TISSUE	0912	0103	11.29	0
	ALU		TISSUE	0793	0098	12.36	0
	ACP	LI	ANTH 67 UG/ML	0874	1181	135.13	0
	ACP	LU	ANTH 67 UG/ML	0489	0876	179.14	0
000298146	ACT	LI1	0158-3 PCT.	0488	0101	20.70	0
000298146	ACT	LI2	0079-3 PCT.	0614	0101	16.45	0
000298146	ACT	LI3	0395-4 PCT.	0397	0086	21.66	0
000298146	ACT	LU1	0158-3 PCT.	0540	0121	22.41	0
000298146	ACT	LU2	0079-3 PCT.	0207	0072	34.78	0
000298146	ACT	LU3	0395-4 PCT.	0497	0112	22.54	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710804 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 07/14/77

COMPOUND	TEST	OPG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1851	0341	0085	18.42	4.59	0
	A-C		SOLVENT	1776	0196	0046	11.04	2.59	0
	ALI		TISSUE	1767	0346	0099	19.58	5.60	0
	ALU		TISSUE	1733	0366	0099	21.12	5.71	0
	ACP	LI	DMN 90 UM/ML	1690	1073	1163	63.49	68.82	0
	ACP	LU	DMN 90 UM/ML	1876	0282	0094	15.03	5.01	0
000298146	ACT	LI1	0033-1 PCT.	1412	0093	0050	6.59	3.54	0
000298146	ACT	LI2	0165-2 PCT.	1866	0234	0066	12.54	3.54	0
000298146	ACT	LI3	0825-3 PCT.	1426	0055	0026	3.86	1.82	0
000298146	ACT	LU1	0033-1 PCT.	0924	0094	0047	10.17	5.09	0
000298146	ACT	LU2	0165-2 PCT.	1694	0245	0081	14.46	4.78	0
000298146	ACT	LU3	0825-3 PCT.	2224	0320	0176	14.39	7.91	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672 DATE - 07/14/77
EXPERIMENT 711801 DETECTOR TA100 SPECIES RHESUS/MONKEY

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP*6	MUT1 EP*0	FREQ1 EP-8	CONTAM
	A*C		DMN 90 UM/ML	0432	0287	66.44	0
	A-C		SOLVENT	0543	0284	52.30	0
	ALI		TISSUE	1742	0599	34.39	0
	ALU		TISSUE	1489	0529	35.53	0
	ACP	LI	DMN 90 UM/ML	0896	0728	81.25	0
	ACP	LU	DMN 90 UM/ML	1930	0671	34.77	0
000298146	ACT	LI1	0158-3 PCT.	1425	0656	46.04	0
000298146	ACT	LI2	0079-3 PCT.	1643	0587	35.73	0
000298146	ACT	LI3	0395-4 PCT.	1829	0567	31.00	0
000298146	ACT	LU1	0158-3 PCT.	1732	0637	36.78	0
000298146	ACT	LU2	0079-3 PCT.	1343	0616	45.87	0
000298146	ACT	LU3	0395-4 PCT.	1565	0551	35.21	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 706803 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0617	0061	9.89	0
	A-C		SOLVENT	0499	0036	7.21	0
	ALI		TISSUE	0845	0076	8.99	0
	ALU		TISSUE	0784	0047	5.99	2
	ACP	LI	DMN 90 UM/ML	0364	0544	149.45	0
	ACP	LU	DMN 90 UM/ML	0824	0087	10.56	0
000298146	ACT	LI1	0158-3 PCT.	0815	0056	6.87	0
000298146	ACT	LI2	0079-3 PCT.	0734	0057	7.77	0
000298146	ACT	LI3	0395-4 PCT.	0744	0051	6.85	0
000298146	ACT	LU1	0158-3 PCT.	0862	0052	6.03	0
000298146	ACT	LU2	0079-3 PCT.	0885	0056	6.33	0
000298146	ACT	LU3	0395-4 PCT.	0913	0094	10.30	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710302 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 07/14/77

COMPOUND	TEST	OPG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1406	0099	7.04	0
	A-C		SOLVENT	1365	0066	4.84	0
	ALI		TISSUE	1700	0144	8.47	0
	ALU		TISSUE	1000	0071	7.10	0
	ACP	LI	AMQ 333 UG/ML	1835	0492	26.81	0
	ACP	LU	AMQ 333 UG/ML	0797	0243	30.49	0
00029R146	ACT	LI1	0158-3 PCT.	1016	0064	6.30	0
00029R146	ACT	LI2	0079-3 PCT.	1062	0060	5.65	0
00029R146	ACT	LI3	0395-4 PCT.	1503	0050	3.33	0
00029R146	ACT	LU1	0158-3 PCT.	1302	0068	5.22	0
00029R146	ACT	LU2	0079-3 PCT.	0629	0050	7.95	0
00029R146	ACT	LU3	0395-4 PCT.	0936	0063	6.73	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672 DATE - 07/14/77
EXPERIMENT 711101 DETECTOR TA1538 SPECIES RHESUS/MONKEY

COMPOUND	TEST	OPG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0555	0103	18.56	0
	A-C		SOLVENT	0565	0085	15.04	3
	ALI		TISSUE	0554	0111	20.04	0
	ALU		TISSUE	0614	0113	18.40	0
	ACP	LI	ANTH 67 UG/ML	0561	1133	201.96	2
	ACP	LU	ANTH 67 UG/ML	0662	0113	17.07	0
000298146	ACT	LI1	0158-3 PCT.	0544	0070	12.87	0
000298146	ACT	LI2	0079-3 PCT.	0597	0048	8.04	1
000298146	ACT	LI3	0395-4 PCT.	0588	0054	9.18	0
000298146	ACT	LU1	0158-3 PCT.	1220	0076	6.23	1
000298146	ACT	LU2	0079-3 PCT.	0617	0093	15.07	1
000298146	ACT	LU3	0395-4 PCT.	0608	0071	11.68	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 708903 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0596	0024	4.03	0
	A-C		SOLVENT	0758	0016	2.11	0
	ALI		TISSUE	0863	0057	6.60	0
	ALU		TISSUE	0868	0033	3.80	0
	ACP	LI	ANTH 67 UG/ML	0760	2055	270.39	0
	ACP	LU	ANTH 67 UG/ML	0815	0042	5.15	0
000298146	ACT	LI1	0158-3 PCT.	0378	0024	6.35	0
000298146	ACT	LI2	0079-3 PCT.	0473	0027	5.71	0
000298146	ACT	LI3	0395-4 PCT.	0485	0029	5.98	0
000298146	ACT	LU1	0158-3 PCT.	0626	0024	3.83	0
000298146	ACT	LU2	0079-3 PCT.	0633	0032	5.06	0
000298146	ACT	LU3	0395-4 PCT.	0770	0024	3.12	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 710904 DETECTOR 0000D4 SPECIES RHESUS/MONKEY DATE - 07/14/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1983	0294	0096	14.83	4.84	0
	A-C		SOLVENT	1451	0150	0036	10.34	2.48	0
	ALI		TISSUE	1231	0190	0159	15.43	12.92	0
	ALU		TISSUE	1537	0283	0126	18.41	8.20	0
	ACP	LI	DMN 90 UM/ML	2163	0789	1228	36.48	56.77	4
	ACP	LU	DMN 90 UM/ML	1999	0324	0112	16.21	5.60	0
000298146	ACT	LI1	0033-1 PCT.	1880	0250	0141	13.30	7.50	0
000298146	ACT	LI2	0165-2 PCT.	2166	0239	0118	11.03	5.45	0
000298146	ACT	LI3	0825-3 PCT.	1927	0247	0148	12.82	7.68	0
000298146	ACT	LU1	0033-1 PCT.	1525	0231	0182	15.15	11.93	0
000298146	ACT	LU2	0165-2 PCT.	1938	0250	0114	12.90	5.88	4
000298146	ACT	LU3	0825-3 PCT.	2418	0150	0152	6.20	6.29	0